## REMARKS

It is respectfully requested that this application be reconsidered in view of the above amendments and the following remarks and that all of the claims under examination be allowed.

### Claim Amendments

Claims 1 and 4 have been amended to recite that the neoplastic cell is a ras-activated neoplastic cell. Support for this recitation can be found, for example, at page 5, line 28. Additionally, step (a) of claim 1 has been amended to recite that the effective amount of a reovirus is intended to increase sensitivity of the ras-activated neoplastic cell to the chemotherapeutic agent. Support for this recitation can be found, for example, at page 18, lines 4-9 and in the original claim 1.

Claims 12, 19, 23 and 25 have been amended to recite that the neoplastic cell is a ras-activated neoplastic cell. Support for this recitation can be found, for example, at page 5, line 28. These claims have further been amended to recite that the proliferative disorder is a ras-mediated proliferative disorder. Support for this recitation can be found, for example, at page 12 lines 18-25. Additionally, these claims have been amended in step (a) to recite that the infected neoplastic cells are neoplastic cells that are refractory to the chemotherapeutic agent. Support for this recitation can be found, for example, at page 11, line 21 and in the original claim 12.

Claims 26 and 30 have been amended to recite that the neoplastic cell is a ras-activated neoplastic cell. Support for this recitation can be found, for example, at page 5, line 28. These claims have been further amended to recite that the resulting infection of the neoplasm by the reovirus prevents development of drug resistance to the chemotherapeutic agent. Support for this recitation can be found, for example, at page 22, lines 8-15 and in the original claim 26.

Accordingly, no new matter has been added by these amendments. The Examiner is hereby requested to enter these amendments.

Applicants submit that all claim amendments presented herein are made solely in the interest of expediting allowance of the claims and should not be interpreted as acquiescence to any rejections or ground of unpatentability. Applicants reserve the right to file at least one continuing application to pursue any subject matter that is canceled or removed from prosecution due to the amendments.

Rejection Under 35 U.S.C. §112, Second Paragraph (Paragraphs 4-6 of the Office Action)

Claims 1-30 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. The rejection is respectfully traversed for the reasons set forth below.

The Office Action states that the term "effective amount" of reovirus and chemotherapeutic agent in claims 1, 12 and 26 is allegedly indefinite as a limitation. Applicants disagree since the term "effective amount" is clearly defined in the specification at page 18, lines 4-9. Particularly, it is disclosed that:

> An "effective amount" is an amount of a chemotherapeutic agent or reovirus which is sufficient to result in the intended effect. For a chemotherapeutic agent used to treat a disease, an efficient amount is an amount sufficient to alleviate or eliminate the symptoms of the disease, or to slow down the progress of the disease. For a reovirus to sensitize a tumor to a chemotherapeutic agent, an efficient amount is an amount sufficient to increase sensitivity to the tumor or the chemotherapeutic agent.

Accordingly, applicants submit the meaning of "effective amount" is clear.

Additionally, claim 1 has been amended in step (a) to recite "an effective amount of a reovirus to increase sensitivity of the ras-activated neoplastic cell to the chemotherapeutic agent". As such, it is clear that an effective amount of reovirus is an amount capable of increasing the sensitivity of the ras-activated neoplastic cells to the chemotherapeutic agent. The same reasoning applies to dependent claims 2-11.

Similarly, claim 12 has been amended in step (a) to recite a method comprising an effective amount of reovirus under conditions that result in infection by the reovirus of the ras-activated neoplastic cells that are refractory to the chemotherapeutic agent. Claim 26 has been amended to recite a method comprising an effective amount of reovirus wherein the infection prevents development of drug resistance to the chemotherapeutic agent.

In sum, the application particularly points out and distinctly claims the subject matter with respect to an "effective amount" of reovirus and chemotherapeutic agent. Therefore, withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. §112, First Paragraph (Paragraphs 8-18 of the Office Action)

Claims 1-6, 8-11, 12-25, 26-28 and 30 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly not being enabled. The rejection is respectfully traversed for the reasons set forth below.

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent, coupled with information known in the art, without undue experimentation. MPEP §2164.01; *United States v. Telectronics, Inc.*, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988).

Per MPEP 2164.01(a), factors to be considered to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue" include but are not limited to:

(A) The breadth of the claims;
(B) The nature of the invention;
(C) The state of the prior art;
(D) The level of ordinary skill;
(E) The level of predictability in the art;
(F) The amount of direction provided by the inventor;
(G) The existence of working examples; and

(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

The determination that "undue experimentation" would have been needed to make and use the claimed invention is not a single, simple factual determination. Rather, it is a conclusion reached by weighing all the above noted factual considerations. *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404. MPEP 2164.01(a).

Applicants respectfully submit that for the reasons discussed below, the rejection lacks foundation since no undue experimentation would be required to make or use the claimed invention.

***Breadth or scope of the claims***

The claimed invention includes methods of sensitizing a ras-activated neoplastic cell to a chemotherapeutic agent, methods of treating ras-activated neoplastic cells that are refractory to a chemotherapeutic agent, and methods for preventing a ras-activated neoplasm from developing drug resistance to a chemotherapeutic agent.

The Office Action alleges that the claims broadly read on a method of sensitizing any or all neoplastic cells to any or all chemotherapeutic agents by reovirus treatment. Furthermore, the Office Action alleges that the claims broadly read on a method for preventing any or all neoplasms from developing drug resistance to any or all chemotherapeutic agents and to a second, any or all chemotherapeutic agent (presumably in reference to claim 30).

Applicants submit that currently amended claims 1, 12 and 26 (as well as the corresponding dependent claims) recite ras-activated neoplastic cells or a ras-activated neoplasm, instead of any neoplastic cells or neoplasm.

***Working examples and amount of guidance in the specification***

The Office Action alleges that the present application does not teach the sensitivities of any or all neoplastic cells to any chemotherapeutic agents other than cisplatin. As discussed above, the claims recite ras-activated neoplastic cells (or ras-activated neoplasms). Furthermore, the specification teaches in a working example (Example 1) that reovirus can be used for increasing the sensitivity of ras-activated neoplastic cells to a representative chemotherapeutic agent, cisplatin. The guidance of Example 1 is enabling for chemotherapeutic agents generally, including the agents recited in claim 6, since it would be a simple matter for a person reasonably skilled in the art to substitute the use of cisplatin for another drug. As disclosed in the application at page 19, line 11,

> Without being limited to a theory, we believe that reovirus sensitizes tumor cells to chemotherapeutic agents by enhancing accumulation of the agents in tumor cells, or by inducing apoptosis. Reovirus is known to inhibit protein synthesis of the host cell in favor of translation of its own proteins. Therefore, reovirus infection may inhibit the synthesis of drug transporter proteins such as MDR1 or the MRPs, and enable drugs to accumulate in the cell. Since drug transporter proteins are responsible for transporting various drugs out of the cell, including structurally unrelated drugs, inhibiting the synthesis of such transporter proteins would lead to sensitization of the cell to a variety of drugs.

The Office Action further alleges that the specification does not teach what the second chemotherapeutic agent is (referencing claim 30 presumably). As discussed above, it is expected that reovirus sensitizes the cells to a variety of chemotherapeutic agents, even structurally unrelated ones. Guidance for chemotherapeutic agents is provided by Example 1 and representative species are disclosed in the specification, for example, at page 13, line 9 to page 14, line 13 and page 20, line 20 to page 21, line 2. Persons having a reasonable level of skill in the art are aware that many standard chemotherapy protocols involve more than one chemotherapeutic agent and the identity of the second chemotherapeutic agent need not be taught specifically.

***State of the art and level of predictability***

The Office Action acknowledges that reovirus oncolysis of a ras-mediated neoplasm is known in the state of the art. The Office Action, however, alleges that because there are many mechanisms by which cancer cells might develop drug resistance, it is unpredictable that any or all drug resistance can be overcome, or that the sensitivity to anti-cancer therapy drugs of a neoplastic cell can be increased, by administering reovirus. As discussed above, the instant claims are directed particularly to administration of reovirus to ras-activated neoplastic cells or ras-mediated neoplasms, rather than neoplastic cells generally. Also as discussed above, it is expected that reovirus sensitizes tumor cells to chemotherapeutic agents by enhancing accumulation of the agents in tumor cells, or by inducing apoptosis. Therefore, it is reasonably predictable that reovirus can be used with respect to a wide variety of chemotherapeutic agents.

In sum, given the state of the art at the time of filing, it was reasonably predictable that reovirus could be used to overcome drug resistance to chemotherapeutic agents in ras-activated neoplastic cells, as well as increase the sensitivity of such cells to chemotherapeutic agents.

***Level of skill in the art***

The Office Action agrees that the level of skill in the art is high.

Accordingly, the scope of the claims is not as broad as alleged by the Office Action, the provided working example and guidance in the specification are enabling and the state of the art supports predictable success. Furthermore, the level of skill in the art is high.

In conclusion, one reasonably skilled in the art could make or use the claimed invention given the disclosure in the application, coupled with information known in the art, without undue experimentation. Therefore, withdrawal of the rejection is respectfully requested.

Rejection Under 35 U.S.C. §112, Written Description (Paragraphs 19-21 of the Office Action)

The rejection of claims, 1-5, 8-11, 12-25, 26-28 and 30 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention, is respectfully traversed for the reasons set forth below.

The Office Action alleges that the specification only describes a method of using reoviruses for increasing the sensitivity of a ras-mediated tumor to cisplatin. The Office Action further alleges that the Applicants did not have possession of a method to increase sensitivity of "any or all neoplastic cells" to "any or all chemotherapeutic drugs" or to "a second chemotherapeutic agent". Applicants disagree.

The written description requirement is satisfied if the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date, applicant was in possession of the invention as now claimed. MPEP §2163.02

With regard to possession of a method to increase sensitivity of "any or all neoplastic cells", the rejection is moot in light of currently amended claims 1, 12 and 26, which now recite ras-activated neoplastic cells.

With regard to possession of a method including "any or all chemotherapeutic drugs", example 1 of the specification describes a method of using reovirus for increasing the sensitivity of ras-activated neoplastic cells to cisplatin. Applicants submit that this disclosure stands as the description of a representative species for any of a number of chemotherapeutic agents. The example was not intended to be limiting, but rather illustrative of the claimed invention. Applicants further submit that the specification discloses a list of representative species of

chemotherapeutic drugs (page 13, line 9 to page14, line 2). Therefore, the specification adequately discloses the invention as currently claimed.

With regard to possession of a method including "a second chemotherapeutic agent", as discussed above, the specification discloses sufficient species of chemotherapeutic agents to represent the genus of chemotherapeutic agents. Any of the chemotherapeutic agents may be the "second chemotherapeutic agent".

The Office Action additionally states, Applicants assume, that the written description requirement has not been met with regard to dosage of the chemotherapeutic drug and reovirus, although the Office Action admits that the dosages are known in the art. Applicants submit that the specification teaches an exemplary injection dosage of cisplatin for mice of 2.5 mg per kilogram of body weight (page 30, line 4). Furthermore, unit dosage forms for reovirus are disclosed in the specification at page 21, line 27 to page 22 line 3 as

> ...containing from about $10^2$ pfus to about $10^{13}$ pfus of the reovirus. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of reovirus calculated to produce the desired effect, in association with a suitable pharmaceutical excipient.

Additionally, an effective amount of both chemotherapeutic and of reovirus are defined in the specification at page 18, lines 4-9.

What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. See *Hybritech Inv. V. Monoclonal Antibodies*, Inc. 802 F.2d at 1384, 231 USPQ at 94. MPEP 2163 II A. 3(a). Applicants submit that the dosages of chemotherapeutic drugs are well known and need not be disclosed in detail. With the exemplary disclosure set forth above, the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date, Applicants were in possession of the invention as claimed.

In view of the above, withdrawal of this rejection is respectfully requested.

Non-statutory Double Patenting Rejections (Paragraphs 22-29 of the Office Action)

A. Copending Application No. 10/602,024

Claims 1-5, 8-11, 12-25, 26-28 and 30 stand provisionally rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 16-22 of copending Application No. 10/602,024 ('024 hereinafter). The rejection is respectfully traversed for the reasons set forth below.

MPEP 804 II B. 1. states:

> A double patenting rejection of the obviousness type is "analogous to [a failure to meet] the nonobviousness requirement of 35 U.S.C. 103" except that the patent principally underlying the double patenting rejection is not considered prior art. *In re Braithwaite*, 379 F.2d 594, 154 USPQ 29 (CCPA 1967). Therefore, any analysis employed in an obviousness-type double patenting rejection parallels the guidelines for analysis of a 35 U.S.C. 103 obviousness determination. *In re Braat*, 937 F.2d 589, 19 USPQ2d 1289 (Fed. Cir. 1991); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985).

MPEP 2143 states:

> "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.
>
> The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

Claim 1 is not obvious in view of the claims of the '024 application. Claim 16 of the '024 application reads:

> 16. A method of treating or ameliorating a ras-activated neoplasm in an animal, comprising:

(a) identifying a ras-activated neoplasm in the animal by providing a group of cells from the animal, contacting the cells with a reovirus under conditions which allow the reovirus to replicate in ras-activated cells, and identifying the cells as comprising ras-activated neoplastic cells if the reovirus can replicate in the cells; and
(b) administering to the animal an effective amount of a therapeutic agent that is selective for ras-activated neoplasms.

Thus, claim 16 of the '024 application requires an ex vivo step of using reovirus to identify a group of cells as ras-activated neoplastic cells, then a second step of treating the ras-activated neoplasm with a therapeutic agent. In contrast, amended claim 1 of the present application involves administering reovirus to ras-activated neoplastic cells to increase their sensitivity to a chemotherapeutic agent.

There is no suggestion or motivation either in the claims of the '024 application or in the knowledge generally available to one of ordinary skill in the art to modify claim 16 of the '024 application to arrive at the invention claimed herein. Specifically, claims of the '024 application do not teach or suggest increasing the sensitivity of cells to chemotherapeutic agents by using reovirus. Nor do the claims of the '024 application provide a reasonable expectation of success that reovirus can be used to increase the sensitivity of ras-activated neoplastic cells to therapeutic agents. Furthermore, the relevant '024 claims do not teach or suggest all the claim limitations of the present invention.

In sum, since none of the three criteria for *prima facie* obviousness have been met, claim 1 is not obvious in view of the claims of the '024 application.

Claim 12 of the present application is directed to treating ras-activated neoplastic cells that are refractory to a chemotherapeutic agent. Claim 26 of the present application is directed to preventing the development of drug resistance to a chemotherapeutic agent in ras-activated neoplastic cells. There is no motivation or suggestion to modify the claims of the '024 application to arrive at the invention of claim 12 or 26 of the present application. No reasonable

expectation of success is provided, and the claims of the '024 application do not include all the claim limitations of claim 12 or 26 of the present application. Accordingly, claim 12 or 26 of the present application is not obvious in view of the claims of the '024 application.

If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); MPEP 2143.03. Since independent claims 1, 12 and 26 are nonobvious under 35 U.S.C. 103, it follows that the remaining rejected claims, which depend from claims 1, 12 or 26, are also nonobvious.

In conclusion, a *prima facie* case of obviousness has not been established, and the requirements under the judicially created doctrine of obviousness-type double patenting are not met. Therefore, withdrawal of the rejection is respectfully requested.

B. U.S. Patent No. 6,565,831

Claims 1-6 and 8-28 stand rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being allegedly unpatentable over claims 1-6, 11-18, 22, 23, 25-28, and 32-34 of U.S. Patent No. 6,565,831 ('831 hereinafter). This rejection is respectfully traversed for the same reasons as set forth above.

Claim 1 of the '831 patent reads:

> 1. A method of treating a ras-mediated neoplasm in a mammal, comprising the steps of:
> a) performing a step selected from the group consisting of:
> i) administering to the neoplastic cells in said mammal an effective amount of an immune suppressive agent;
> ii) removing anti-reovirus antibodies from said mammal;
> iii) administering anti-antireovirus antibodies to said mammal; and
> iv) suppressing the immune system of the mammal; and
> b) administering to the neoplastic cells in said mammal an effective amount of one or more reoviruses under conditions which result in substantial lysis of the neoplastic cells.

This claim, or its dependent claims, does not teach or suggest that reovirus increases the sensitivity of ras-activated cells to chemotherapeutic agents, that reovirus can render ras-activated neoplastic cells refractory to a chemotherapeutic agent, or that reovirus prevents the development of drug resistance to a chemotherapeutic agent in ras-activated neoplastic cells. Furthermore, there is no motivation or suggestion to modify the claims of the '831 patent to arrive at the presently claimed invention. The requirements of reasonable expectation of success and inclusion of all the claim limitations are also not satisfied.

Since the requirements under the judicially created doctrine of obviousness-type double patenting are not met, withdrawal of the rejection is respectfully requested.

C. U.S. Patent No. 6,136,307

Claims 1-6 and 8-28 also stand rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1, 3-8, 13-20, 24-33 and 34 of U.S. Patent No. 6,136,307 ('307 hereinafter). This rejection is respectfully traversed for the reasons set forth below.

Claim 1 of the '307 patent reads:

> 1. A method of treating a ras-mediated proliferative disorder in a mammal suffering from said disorder, wherein said mammal is selected from the group consisting of dogs, cats, sheep, goats, cattle, horses, pigs, humans and non-human primates, and wherein said method comprises administering to said mammal an effective amount of at lest one reovirus in the absence of BCNU under conditions which result in substantial lysis of the ras-mediated proliferating cells in said mammal.

Claim 27 of the '307 patent reads:

> 27. The method of claim 1 further comprising the administration of an effective amount of a chemotherapeutic agent, with the proviso that the chemotherapeutic agent is not BCNU.

However, claims of the '307 patent do not teach or suggest that reovirus increases the sensitivity of ras-activated cells to chemotherapeutic agents, that reovirus can render ras-activated neoplastic cells refractory to a chemotherapeutic agent, or that reovirus prevents the development of drug resistance to a chemotherapeutic agent in ras-activated neoplastic cells. Again, the presently claimed invention is not obvious in view of the claims of the '307 patent.

Accordingly, withdrawal of the rejection is respectfully requested.

Rejection Under 35 U.S.C. §102 (Paragraphs 31-41 of the Office Action)

A. Robert et al. (WO 99/18799)

The rejection of claims 1-6, 8, 9, 12-14, 17-23, 26-28 under 35 U.S.C. §102(b) as allegedly being anticipated by Roberts et al. (WO 99/18799; "Roberts" hereinafter) is respectfully traversed for the reasons set forth below.

The standard of anticipation under 35 U.S.C. §102 is that each and every element of the claim must be found in the cited reference. *In re Marshall*, 198 USPQ 344 (CCPA 1978).

Roberts does not teach each and every element of the claimed invention. Roberts teaches a method of treating a neoplasm in a mammal comprising administration of an interferon-sensitive virus into a mammal suffering a neoplasm. On the other hand, currently amended claim 1 and its dependent claims are drawn to a method of sensitizing a ras-activated neoplastic cell to a chemotherapeutic agent using reovirus. Since Roberts does not teach either treatment of ras-activated neoplastic cells or increasing such cells' sensitivity to chemotherapeutic agents by administering reovirus, Roberts does not teach each and every element of the claimed invention. Therefore, claim 1, or its dependent claims 2-6, 8 or 9, is not anticipated by this reference.

Currently amended claim 12 and its dependent claims recite a method of treating a subject harboring a ras-mediated proliferative disorder wherein said subject comprises ras-activated neoplastic cells that are refractory to a chemotherapeutic agent, comprising the steps of: administering to the subject an effective amount of reovirus under conditions that result in infection by the reovirus of the ras-activated neoplastic cells that are refractory to the chemotherapeutic agent; and administering an effective amount of chemotherapeutic agent to said subject. Since the Roberts application does not teach infection of ras-activated neoplastic cells that are refractory to a chemotherapeutic agent, the reference does not teach each and every element of the claimed invention. As such, this reference does not anticipate claim 12 or its dependent claims.

Currently amended claim 26 and its dependent claims recite a method for preventing a ras-activated neoplasm in a subject from developing drug resistance to a chemotherapeutic agent, comprising the steps of: administering to the subject an effective amount of reovirus under condition which result in infection of the ras-activated neoplasm by the reovirus; and administering to the subject an effective amount of a chemotherapeutic agent wherein the infection prevents development of drug resistance to the chemotherapeutic agent. Since Roberts does not teach that reovirus infection of ras-activated neoplastic cells prevents development of drug resistance to a chemotherapeutic agent, the reference does not teach each and every element of the claimed invention. As such, claim 26, or its dependent claim 27 or 28, is not anticipated by this reference.

B. Mercer University

The rejection of claims 1, 4, 8, 12, 17, 19, 20, 22 and 26 under 35 U.S.C. §102(b) as allegedly being anticipated by Mercer University (Mercer University Home page 1996, pp. 1-2; "Mercer" hereinafter) is respectfully traversed for the reasons set forth below.

Mercer teaches that the combination of reovirus type 3 and a chemotherapeutic compound BCNU resulted in a 100% reduction of implanted tumors in mice. Mercer does not teach the treatment of ras-activated tumors particularly. Furthermore, Mercer does not teach sensitizing a ras-activated neoplastic cell to a chemotherapeutic agent (claim 1), treating ras-activated neoplastic cells that are refractory to a chemotherapeutic agent (claim 12), or preventing a ras-activated neoplasm from developing drug resistance to a chemotherapeutic agent (claim 26). In sum, Mercer fails to teach each and every limitation found in currently amended claim 1, 12 or 26. Since claims dependent from claim 1, 12 or 26 contain all the elements of their respective base claims, Mercer also does not teach each and every element of the dependent claims.

C. Williams et al.

The rejection of claims 1, 4, 8, 12, 17, 19, 20, 22 and 26 under 35 U.S.C. §102(b) as allegedly being anticipated by Williams et al. (Cancer Immunol. Immunother. 1986, Vol. 23 (2), pp. 87-92; "Williams" hereinafter) is respectfully traversed for the reasons set forth below.

Williams teaches a method of treating L1210 neoplastic cells with a chemotherapeutic compound BCNU and reovirus *in vitro* to inhibit tumor cell proliferation and growth. L1210 cells are not ras-activated neoplastic cells (see, e.g. Umezawa et al. (1996), copy enclosed herewith). Therefore, Williams does not teach the treatment of ras-activated neoplastic cells or the administration of reovirus to such cells. Furthermore, Williams does not teach sensitizing a ras-activated neoplastic cell to a chemotherapeutic agent (claim 1), treating ras-activated neoplastic cells that are refractory to a chemotherapeutic agent (claim 12), or preventing a ras-activated neoplasm from developing drug resistance to a chemotherapeutic agent (claim 26). In sum, Williams fails to teach each and every limitation found in claim 1, 12, 26 or their dependent claims.

D. US Patent No. 6,136,307 and PCT Publication No. WO 00/50051

The rejection of claims 1-6, 8, 9, 12-14, 17-23, and 26-28 under 35 U.S.C. §102(a) as allegedly being anticipated by US Patent No. 6,136,307 ('307 hereinafter) or PCT Publication No. WO 00/50051 of Lee et al. ("Lee" hereinafter) is respectfully traversed for the reasons set forth below.

The '307 patent and Lee teach a method of treating a ras-mediated proliferation disorder in a mammal comprising administration of a reovirus, and optionally a chemotherapeutic agent, into a mammal suffering a proliferative disorder. Neither the '307 patent nor Lee specifically teaches a method of sensitizing a ras-activated neoplastic cell to a chemotherapeutic agent (claim 1), treating ras-activated neoplastic cells that are refractory to a chemotherapeutic agent (claim 12), or preventing a ras-activated neoplasm from developing drug resistance to a chemotherapeutic agent (claim 26). As discussed above, either reference fails to teach each and every element of the claimed invention.

In sum, none of the Roberts, Mercer, Williams, '307 and Lee references teaches each and every element of the claimed invention. Accordingly, the requirement under 35 U.S.C. §102 is not met, and withdrawal of the rejections is respectfully requested.

Conclusions

For the reasons set forth above, Applicants submit that the claims of this application are patentable. Reconsideration and withdrawal of the Examiner's rejections are hereby requested. Allowance of the claims under examination in this application is earnestly solicited.

In the event that a telephone conversation could expedite the prosecution of this application, the Examiner is requested to call the undersigned at (650) 839-5006.

Please apply any charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 8-17-04

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DRUGS EXPTL. CLIN. RES. **XXII** (2) 35–40 (1996)

# GROWTH INHIBITION OF K-*RAS*-EXPRESSING TUMOURS BY A NEW VINCA ALKALOID, CONOPHYLLINE, IN NUDE MICE

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2) Department of Surgery, Tokyo Metropolitan Komagome Hospital, Honkomagome, Tokyo 113.
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**Summary:** *Conophylline, a new vinca alkaloid isolated from the plant* Ervatamia microphylla *induced normal flat morphology in K-*ras*-NRK and K-*ras*-NIH cell lines, and lowered the increased uptake of 2-deoxyglucose in K-*ras*-NRK cells. Conophylline inhibited the growth of K-*ras*-NRK cells, but this inhibition was reversible. The alkaloid also inhibited the growth of K-*ras*-NRK and K-*ras*-NIH3T3 tumours transplanted into nude mice. On the other hand, it showed no effect on survival of the mice loaded with L1210 leukaemia. Thus, conophylline is a new antitumour vinca alkaloid that induced normal phenotypes in* ras*-expressing cells.*

## Introduction

Activation of the *ras* proto-oncogene is found in about 20% of all human neoplasms. The incidence is especially high in pancreatic (81%), bile duct (67%), and colon (41%) carcinomas [1], which cancers are difficult to treat by present chemotherapy. Therefore, *ras*-function inhibitors may help to suppress these tumours when they are used solely or in combination with other anticancer drugs. Oxanosine [2] and compactin [3] were reported as *ras* function inhibitors, but their activities appear to be limited, since they induce normal phenotypes in K-*ras*$^{ts}$-NRK cells but not in K-*ras*-NRK cells.

Recently, we isolated a new vinca alkaloid as a *ras* function inhibitor from the leaves of *Ervatamia microphylla* collected in Thailand [4]. This alkaloid, which we called III-121C, was shown to be identical with conophylline (Fig. 1), a vinca alkaloid

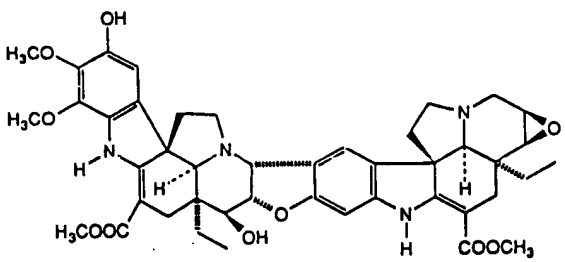


**Fig. 1** Conophylline.

* *Author to whom correspondence should be addressed.*

0378-6501/96/2/00035 + 6 $02.50/0

**Fig. 2** Induction of flat conversion by conophylline in K-*ras*-NRK and K-*ras*-NIH3T3 cells. K-*ras*-NRK cells were incubated with none (A) or 0.3 μg/ml of conophylline (B) for 24 h. K-*ras*-NIH3T3 cells were incubated with none (C) or 0.3 μg/ml of conophylline (D) for 24 h.

recently isolated from *Tabernaemontana divaricata* of Malaysia [5]. Conophylline induced normal flat morphology in K-*ras*$^{ts}$-NRK cells at the permissive temperature, but at the non-permissive temperature it did not induce any marked morphological change [4].

In the present study we examined the effect of conophylline on the growth properties of K-*ras*-NRK cells *in vitro* and *in vivo*. We demonstrated its antitumour activity in mice toward K-*ras*-NRK and K-*ras*-NIH3T3 tumours.

## Materials and methods

*Materials*. Conophylline was isolated from *Ervatamia microphylla* as previously described [2]. K-*ras*-NRK cells were obtained from Dr. M. Hori, Showa College of Pharmaceutical Sciences, Tokyo. K-*ras*-NIH3T3 cells were obtained from Dr. T. Takenaka, University of Tokyo. NRK and NIH3T3 cells were procured from Flow Laboratories and the Japanese Cancer Research Cell Resources Bank, respectively.

*Cell culture*. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% calf serum at 37°C in a 5% $CO_2$/95% air atmosphere. For the morphological assay, cells were incubated at $3 \times 10^4$/well in 1 ml of medium in a 12-well plate for one day, and the chemical was then added. For the cell growth assay, the cells were plated at $1.5 \times 10^4$/well in a 48-well plate in 0.5 ml of medium for one day, after which the chemical was added.

*2-Deoxyglucose transport assay*. Glucose transport assay was carried out as described by Kozma *et al.* [6] with slight modifications. K-*ras*-NRK or NRK cells were inoculated at $3 \times 10^4$ cells/well in the collagen-coated 12-well plates. After two days conophylline was added and the cells were incubated for indicated periods. Then, the medium was replaced by 0.5 ml of phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 2-[$^3$H]-deoxyglucose (0.5 μCi/well, 0.1 mM, American Radiolabeled Chemicals, USA) and the cells were incubated for 10 min at 37°C. The cells were washed with cold PBS and solubilized by 1% SDS. The non-specific value was obtained by addition of 20 μM cytochalasin B, an inhibitor of the glucose transporter.

In vivo *xenograft assay*. Five-week-old female athymic nude mice (Balbc/nu nu) were purchased from Nihon Kurea, Tokyo and bred in an aseptic room. Tumour cells ($10^7$) were inoculated into a subcutaneous cavity, and the intramuscular administration of conophylline was started when the tumour had become 3–4 mm in diameter. The chemical was dissolved in a trace of ethanol and added to olive oil; then 0.1 ml of the mixture was given to

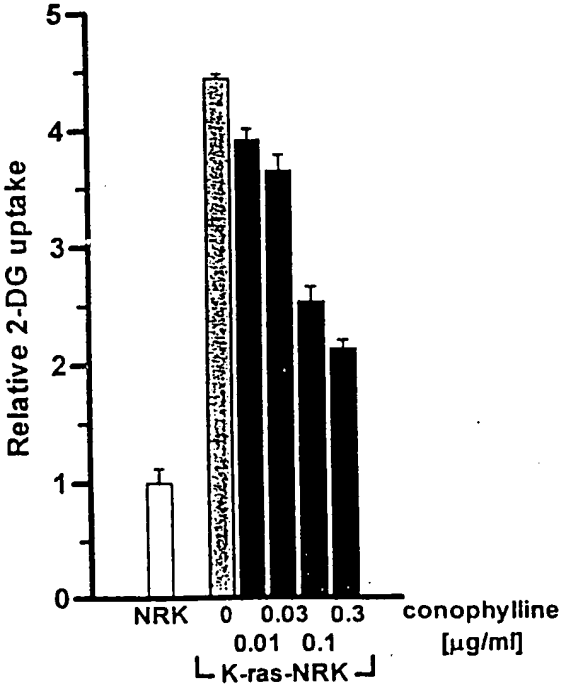


**Fig. 3** Inhibition of 2-deoxyglucose transport by conophylline in K-*ras*-NRK cells. K-*ras*-NRK cells were incubated with conophylline for 24 h and then assayed for glucose transport as described in **Materials and methods**. Each value is mean ± s.d. of triplicate determinations.

mice every other day. The tumour volume was measured with calipers every day.

## Results

Addition of conophylline at 0.3 µg/ml induced flattening of K-*ras*-NRK and K-*ras*-NIH3T3 cells within one day, as shown in Fig. 2. Oxanosine and lovastatin were totally inactive to induce normal morphology in these cell lines. The morphological change by conophylline was reversible, for after the removal of the chemical the flat morphology was lost within two days (data not shown). K-*ras*-NRK cells showed higher glucose uptake activity than NRK cells as previously reported [7]. As shown in Fig. 3, the increased uptake was lowered by incubation with conophylline at the similar concentration to induce flat morphology. A similar period of time (24 h) was required for inhibition of glucose uptake and morphological change.

Conophylline inhibited the growth of K-*ras*-NRK and K-*ras*-NIH3T3 with $IC_{50}$s of 0.25 and 0.40 µg/ml, respectively. As shown in Fig. 4, 0.3 µg/ml of conophylline inhibited the growth of K-*ras*-NRK cells almost completely. However, after the removal of the chemical the cells began to grow; therefore, inhibition of the growth by conophylline was also reversible.

Finally, we tested the *in vivo* antitumour activity against *ras*-expressing tumours in mice. The K-*ras*-NRK tumour grew extremely rapidly in nude mice,

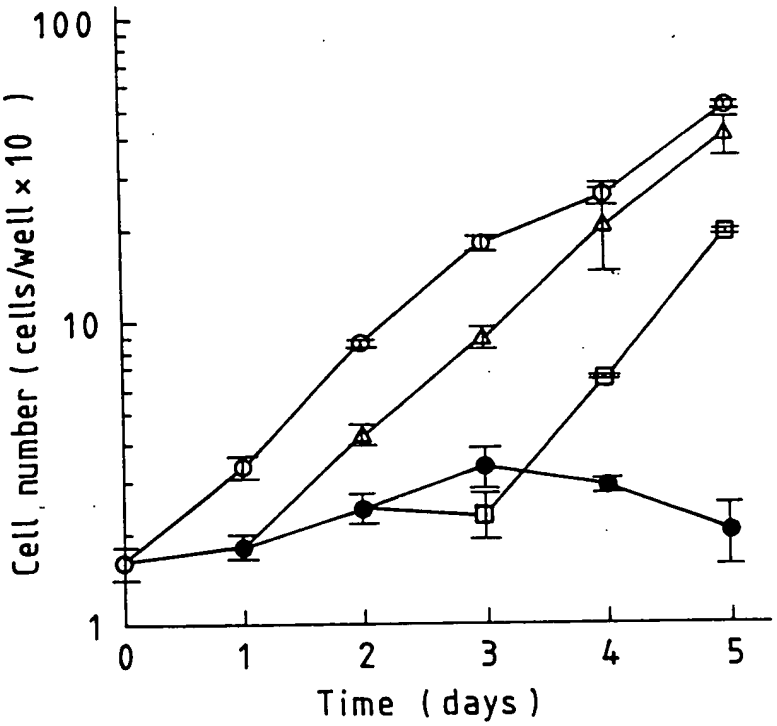


**Fig. 4** Reversible Inhibition of K-*ras*-NRK cell growth by conophylline. The cells were incubated with (•) or without (○) 0.3 µg/ml of conophylline. Conophylline was removed on day 1 (△) or day 2 (□). Each value is mean ± s.d. of triplicate determinations.

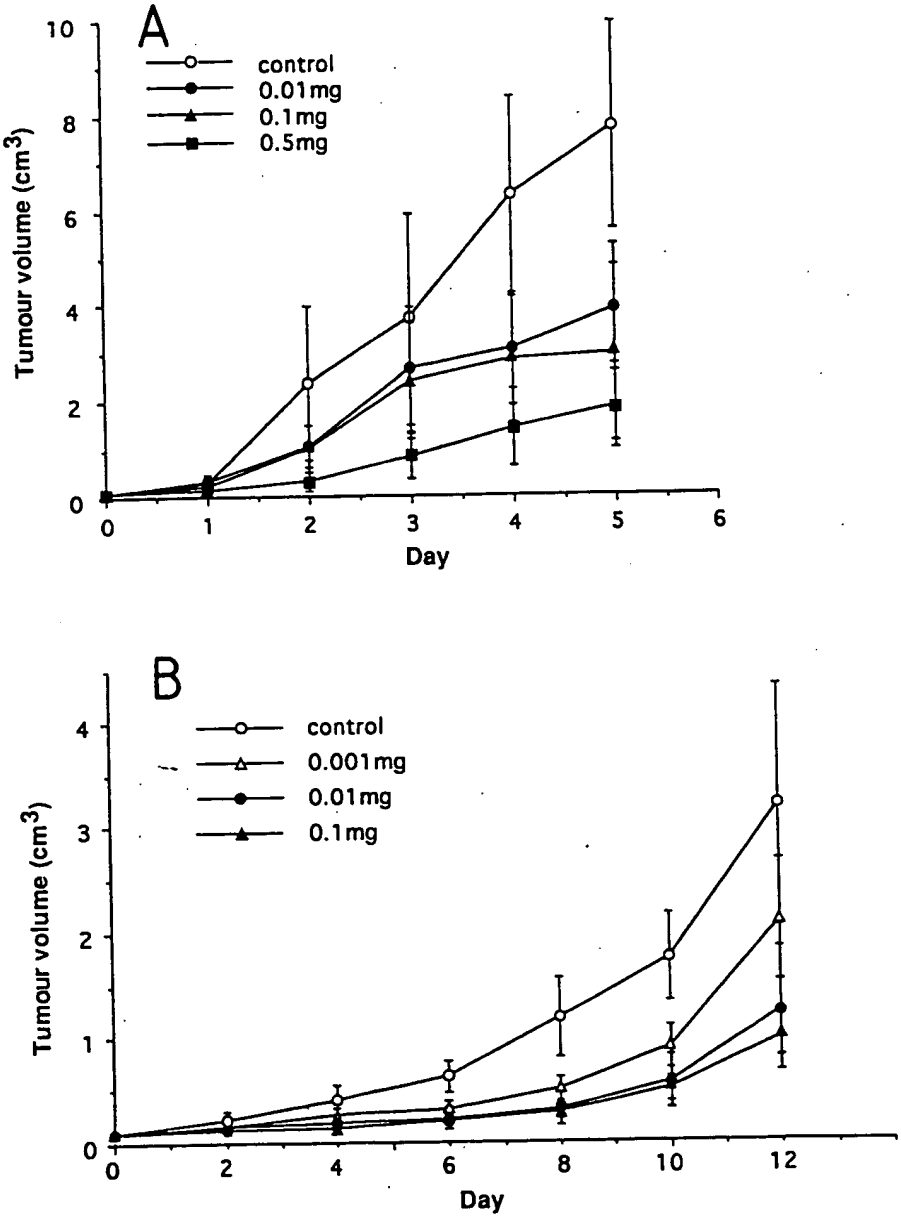


**Fig. 5** Inhibition of K-*ras*-expressing tumour growth by conophylline in nude mice. Tumour cells ($1 \times 10^7$) were transplanted into a subcutaneous cavity of nude mice. When the tumour was 3–4 mm in diameter (day 0), conophylline was intramuscularly given to mice every other day on days 0, 2, 4 ···. Each group consisted of six mice. (A) K-*ras*-NRK tumour (B) K-*ras*-NIH3T3 tumour.

and the volume reached about 8 cc in five days. Intramuscular administration of conophylline at 0.01–0.5 mg/mouse inhibited the growth of the tumour dose-dependently, as shown in Fig. 5A. Conophylline at 0.5 mg/mouse was toxic to the animals, inducing their depressed movement. The K-*ras*-NIH3T3 tumour grew more slowly than the K-*ras*-NRK tumour. Conophylline also inhibited the growth of K-*ras*-NIH3T3 tumour at 0.001–0.1 mg/mouse (Fig. 5B) without any toxicity. On the other hand, conophylline did not increase the survival days of mice loaded with L1210 leukaemia, although it inhibited the growth *in vitro* with an $IC_{50}$ of 0.34 μg/ml. L1210 cells ($1 \times 10^5$ cells/mouse) were inoculated in the peritoneal cavity on day 0, and the chemical was given intraperitoneally. Each group consisted of five female $CDF_1$ mice aged about six weeks. The mean survival period was about eight days in control, and conophylline given at 0.03–0.3 mg/mouse on either (days 1, 4), (days 1, 3, 5, 7, 9) or (days 1–3, 5–7, 9–11) did not significantly increase the survival.

## Discussion

Vinca alkaloids such as vinblastine and vincristine are widely used as anticancer drugs. They are microtubule blockers and inhibit nuclear spindle formation, thus inhibiting normal mitosis. Although a vinca alkaloid, conophylline has a considerably different cellular effect than vinblastine, since the former induces a G1 block (unpublished results) while the latter induces a G2/M block in cycling cells [8]. Lovastatin that inhibits *ras* function by inhibiting farnesyl synthesis also induces G1 block [9]. It is not clear whether conophylline inhibits *ras* function specifically or other G1 components. The molecular target of conophylline is now under study.

Conophylline does not only induce flat morphology in *ras*-expressing cells, but also reverses the increased glucose uptake. Decrease of glucose uptake may contribute to lowering the growth ability in *ras*-expressing cells. Conophylline shows antitumour effect on *ras*-expressing tumours in mice at comparatively low doses: therefore, its distribution and stability in the body may be favourable. The antitumour effect of conophylline on other *ras*-expressing and non-expressing tumours is now under study.

## Acknowledgements

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